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IS/ISO 5983-1 (2005): Animal Feeding stuffs - Determination of nitrogen content and calculation of crude protein content, Part 1: Kjeldahl method [FAD 5: Livestock Feeds, Equipment and Systems]



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प्रोटीन की मात्रा मापन

भाग 1 जैलडाल पद्धति

Indian Standard

**ANIMAL FEEDING STUFFS — DETERMINATION OF
NITROGEN CONTENT AND CALCULATION OF
CRUDE PROTEIN CONTENT**

PART 1 KJELDAHL METHOD

ICS 65.120

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NATIONAL FOREWORD

This Indian Standard (Part 1) which is identical with ISO 5983-1 : 2005 'Animal feeding stuffs — Determination of nitrogen content and calculation of crude protein content — Part 1: Kjeldahl method' issued by the International Organization for Standardization (ISO) was adopted by the Bureau of Indian Standards on the recommendation of the Livestock Feeds, Equipment and Systems Sectional Committee and approval of the Food and Agriculture Division Council.

The text of ISO Standard has been approved as suitable for publication as an Indian Standard without deviations. Certain conventions are, however, not identical to those used in Indian Standards. Attention is particularly drawn to the following:

- a) Wherever the words 'International Standard' appear referring to this standard, they should be read as 'Indian Standard'.
- b) Comma (,) has been used as a decimal marker in the International Standard, while in Indian Standards, the current practice is to use a point (.) as the decimal marker.

In this adopted standard, reference appears to the following International Standard for which Indian Standard also exists. The corresponding Indian Standard, which is to be substituted in its place, is listed below along with its degree of equivalence for the edition indicated:

<i>International Standard</i>	<i>Corresponding Indian Standard</i>	<i>Degree of Equivalence</i>
ISO 6498 : 1998 Animal feeding stuffs — Preparation of test samples	IS 14831 : 2000 Animal feeding stuffs — Preparation of test samples	Identical

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'.

*Indian Standard***ANIMAL FEEDING STUFFS — DETERMINATION OF
NITROGEN CONTENT AND CALCULATION OF
CRUDE PROTEIN CONTENT****PART 1 KJELDAHL METHOD****1 Scope**

This part of ISO 5983 specifies a method for the determination of the nitrogen content of animal feeding stuffs by the Kjeldahl process, and a method for the calculation of the crude protein content.

The method does not measure oxidized forms of nitrogen or heterocyclic nitrogen compounds.

This method does not distinguish between protein nitrogen and non-protein nitrogen. If it is important to determine the content of non-protein nitrogen, an appropriate method should be used.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 6498, *Animal feeding stuffs — Preparation of test samples*

3 Principle

The organic matter is digested by sulfuric acid in the presence of a catalyst. The reaction product is rendered alkaline, then the liberated ammonia is distilled and titrated. The nitrogen content is calculated and the result is multiplied by the conventional factor to obtain the crude protein content.

4 Reagents and materials

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or deionized water or water of equivalent purity.

The reagents [except the standard materials (4.6)] shall be practically free from nitrogenous compounds.

4.1 Potassium sulfate.**4.2 Catalyst, either 4.2.1 or 4.2.2.****4.2.1 Copper(II) oxide (CuO).****4.2.2 Copper(II) sulfate pentahydrate (CuSO₄·5H₂O).****4.3 Sulfuric acid, $c(\text{H}_2\text{SO}_4) = 18 \text{ mol/l}$, $\rho_{20}(\text{H}_2\text{SO}_4) = 1,84 \text{ g/ml}$.**

4.4 Paraffin wax.

4.5 Saccharose.

4.6 Standard materials, either 4.6.1 or 4.6.2.

4.6.1 Acetanilide, with melting point 114 °C; nitrogen (N) content 103,6 g/kg.

4.6.2 Tryptophan, with melting point 282 °C; nitrogen (N) content 137,2 g/kg.

Dry before use.

4.7 Sodium hydroxide solution, $w(\text{NaOH}) = 33\%$ (mass fraction).

4.8 Collecting liquid, either 4.8.1 or 4.8.2.

4.8.1 Sulfuric acid, standard volumetric solution, $c(\text{H}_2\text{SO}_4) = 0,05\text{ mol/l}$ or $c(\text{H}_2\text{SO}_4) = 0,125\text{ mol/l}$.

4.8.2 Boric acid, $\rho(\text{H}_3\text{BO}_3) = 40\text{ g/l}$.

4.9 Solutions for titration.

4.9.1 Sodium hydroxide, standard volumetric solution, $c(\text{NaOH}) = 0,1\text{ mol/l}$ or $c(\text{NaOH}) = 0,25\text{ mol/l}$.

4.9.2 Sulfuric acid, standard volumetric solution, $c(\text{H}_2\text{SO}_4) = 0,05\text{ mol/l}$ or $c(\text{H}_2\text{SO}_4) = 0,125\text{ mol/l}$.

The molarity of standard volumetric solutions should be known to the fourth decimal point.

4.10 Mixed indicator, neutral point at pH 4,4 to 5,8.

Dissolve 2 g of methyl red and 1 g of methylene blue in 1 000 ml of ethanol [$\varphi(\text{C}_2\text{H}_5\text{OH}) = 95\%$ (volume fraction)].

4.11 pH indicator paper.

4.12 Boiling aids, such as granulated pumice stone, or glass beads of diameter 5 mm to 7 mm, or carborundum chips, washed in hydrochloric acid and in distilled water, and ashed.

5 Apparatus

Usual laboratory apparatus and, in particular, the following.

5.1 Analytical balance.

5.2 Digestion, distillation and titration apparatus.

6 Sampling

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage.

Sampling is not part of the method specified in this part of ISO 5893. A recommended sampling method is given in ISO 6497.

Store the sample in such a way that deterioration and change in its composition are prevented.

7 Preparation of test sample

Prepare the test sample in accordance with ISO 6498.

8 Procedure

WARNING — The operations described in 8.3.1 and 8.3.2 should be carried out under a well-ventilated hood or in a fume cupboard which is resistant to sulfuric acid.

8.1 General

For general directions on the application of the Kjeldahl method, see ISO 1871.

8.2 Test portion

Weigh, to the nearest 1 mg, a mass of the test sample chosen according to the expected nitrogen content so that the test portion contains between 0,005 g and 0,2 g of nitrogen and, preferably, more than 0,02 g.

The mass of the test portion of homogeneous air-dry samples should be between 0,5 g and 2,0 g. The mass of the test portion of wet and/or inhomogeneous samples should be between 2,5 g and 5,0 g.

8.3 Determination

8.3.1 Digestion of organic matter

Transfer the test portion quantitatively into a Kjeldahl digestion flask of suitable size (usually 800 ml).

Add 15 g of potassium sulfate (4.1).

Add an appropriate quantity of catalyst as follows: 0,3 g of copper(II) oxide (4.2.1) or 0,9 g to 1,2 g of copper(II) sulfate pentahydrate (4.2.2).

Add 25 ml of sulfuric acid (4.3) for the first gram of dry matter of the test portion and 6 ml to 12 ml for each additional gram of dry matter. Mix thoroughly, ensuring complete wetting of the test portion.

Support the flask so that its axis is inclined at an angle of 30° to 45° to the vertical. Maintain the flask in this position throughout heating.

Heat the flask moderately at first to prevent foam from rising into the neck of the flask or escaping from the flask.

NOTE 1 It may be advisable to add an anti-foaming agent such as paraffin wax (4.4).

Heat moderately, swirling from time to time, until the mass has carbonized and the foam has disappeared. Then heat more intensively until the liquid is boiling steadily.

NOTE 2 Heating is adequate if the boiling acid condenses towards the middle of the neck of the Kjeldahl flask.

Avoid overheating of the walls of the flask not in contact with liquid.

NOTE 3 If a naked flame is used, such overheating can be prevented by placing the flask on a sheet of heat-resistant material with an aperture of diameter slightly less than that of the flask at the liquid level.

After the liquid has become clear with a light green-blue colour, heat for another 2 h.

Leave to cool. If the digest starts to solidify, add some water and mix by swirling.

8.3.2 Distillation of ammonia

8.3.2.1 Carefully add 250 ml to 350 ml of water to dissolve the sulfates completely. If necessary, facilitate dissolving by heating the flask in warm water. Mix by swirling and allow to cool.

Add a few boiling aids (4.12).

For some specific samples, the sulfates may not completely dissolve in the added water. In that case, it is recommended to repeat the digestion with a reduced mass of potassium sulfate (4.1).

8.3.2.2 Pipette, into the collecting flask of the distillation apparatus, 25 ml of the sulfuric acid (4.8.1), choosing the concentration according to the expected nitrogen content of the test portion. Add 100 ml to 150 ml of water. Add a few drops of the mixed indicator (4.10). Proceed in accordance with 8.3.2.4.

8.3.2.3 Alternatively, transfer into the collecting flask 100 ml to 250 ml of boric acid (4.8.2). Add a few drops of mixed indicator (4.10).

Simultaneous titration of the ammonia (see 8.3.3.3) during distillation is recommended since it facilitates verification of the end of distillation.

8.3.2.4 Immerse the end of the condenser in the liquid contained in the collecting flask, to a depth of at least 1 cm.

Slowly pour 100 ml of sodium hydroxide solution (4.7) into the digestion flask along the wall.

Immediately connect the flask to the distillation apparatus.

Heat the flask in such a manner that approximately 150 ml of distillate is collected in 30 min. At the end of this time, check the pH of the distillate at the tip of the condenser using litmus paper (4.11). If the reaction is alkaline, continue distillation.

IMPORTANT — Lift the condenser from the liquid just before the end of the distillation, to prevent backflow.

If, during distillation using sulfuric acid as collecting liquid, the contents of the collecting flask become alkaline, recommence the determination, making appropriate adjustments.

8.3.3 Titration

8.3.3.1 Titration with automatic endpoint indication using a pH-meter is recommended. Otherwise, the endpoint is indicated by the change in colour of the mixed indicator (4.10) added in 8.3.2.

8.3.3.2 If sulfuric acid is used as the collecting liquid, titrate, in the collecting flask, the excess sulfuric acid with sodium hydroxide solution (4.9.1), $c(\text{NaOH}) = 0,1 \text{ mol/l}$ or $c(\text{NaOH}) = 0,25 \text{ mol/l}$ as appropriate, until the endpoint is indicated by the pH-meter or until the colour changes from violet to green.

8.3.3.3 If boric acid is used as the collecting liquid, titrate the ammonia with sulfuric acid (4.9.2), $c(\text{H}_2\text{SO}_4) = 0,05 \text{ mol/l}$ or $c(\text{H}_2\text{SO}_4) = 0,125 \text{ mol/l}$ as appropriate, until the endpoint is indicated by the pH-meter or the colour changes from green to violet.

If simultaneous titration is not possible (see 8.3.2.3), the titration should be carried out as soon as possible after the distillation is complete, ensuring that the temperature of the distillate does not exceed 25 °C. Under these conditions, losses of ammonia are avoided.

8.4 Blank test

Perform a blank test using about 1 g of saccharose (4.5) in place of the test portion.

8.5 Check test

Perform a check test by determining the nitrogen content of acetanilide (4.6.1) or tryptophan (4.6.2) after addition of 1 g of saccharose (4.5).

The choice of the substance for the check test should be related to the digestibility of the samples to be analysed. Acetanilide is easily digested, whereas the digestion of tryptophan is more difficult.

The recovery of nitrogen from acetanilide or tryptophan should be at least 99,5 % for acetanilide and at least 99,0 % for tryptophan.

9 Calculation and expression of results

9.1 Calculation of nitrogen content

9.1.1 Distillate collected in sulfuric acid

Provided that the volumes of sulfuric acid used to collect the ammonia for the determination (8.3) and for the blank test (8.4) are equal, calculate the nitrogen content, w_{n1} , in grams per kilogram of the test sample, by the following equation:

$$w_{n1} = \frac{(V_0 - V_1) \times c_1 \times M}{m}$$

where

V_0 is the volume, in millilitres, of the sodium hydroxide solution (4.9.1) required for the blank test;

V_1 is the volume, in millilitres, of the sodium hydroxide solution (4.9.1) required for the determination;

c_1 is the concentration, in moles per litre, of the sodium hydroxide solution (4.9.1) used for the titrations;

M is the molar mass, in grams per mole, of nitrogen ($M = 14$ g/mol);

m is the mass, in grams, of the test portion.

Report the result to the nearest 0,01 g/kg.

9.1.2 Distillate collected in boric acid

Calculate the nitrogen content of the test sample by the equation:

$$w_{n2} = \frac{2(V_3 - V_2) \times c_2 \times M}{m}$$

where

w_{n2} is the nitrogen content, in grams per kilogram, of the test sample;

V_2 is the volume, in millilitres, of the sulfuric acid (4.9.2) required for the blank test;

V_3 is the volume, in millilitres, of the sulfuric acid (4.9.2) required for the determination;

M is the molar mass, in grams per mole, of nitrogen ($M = 14$ g/mol);

c_2 is the concentration, in moles per litre, of the sulfuric acid (4.9.2) used for the titrations;

m is the mass, in grams, of the test portion.

Report the result to the nearest 0,01 g/kg.

9.1.3 Calculation of crude protein content

The crude protein content may be reported in percent or in grams per kilogram. Calculate the crude protein content of the test sample:

$$w_n = 6,25 \ w_p \text{ g/kg}$$

or

$$w_p = 0,625 \ w_n \ %$$

where

w_p is the crude protein content, expressed in grams per kilogram or in percent;

w_n is the nitrogen content, in grams per kilogram, of the test sample (either w_{n1} or w_{n2} , see 9.1);

Report the result to the nearest 0,01 g/kg or 0,1 %.

10 Precision

10.1 Interlaboratory test

Details of an interlaboratory test on the precision of the method are summarized in Annex A. The values derived from this interlaboratory test may not be applicable to concentration ranges and matrices other than those given.

10.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, will in not more than 5 % of cases be greater than the repeatability limit (r) derived from the equation:

$$r = 0,3 \ \% + 0,008 \ w_p$$

where

r is the repeatability limit, in percent;

w_p is the mean of the two single test results for crude protein content, in percent.

10.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases be greater than the reproducibility limit (R) derived from the equation:

$$R = 1,3 \ \% + 0,027 \ w_p$$

where

R is the reproducibility limit, in percent;

w_p is the mean of the two single test results for crude protein content, in percent.

11 Test report

The test report shall specify:

- a) all information necessary for the complete identification of the sample;
- b) the sampling method used, if known;
- c) the test method used, with reference to this part of ISO 5983;
- d) all operating details not specified in this part of ISO 5983, or regarded as optional, together with details of any incidents occurred when performing the method, which may have influenced the test result(s);
- e) the test result obtained, either the nitrogen content, or the crude protein content, in grams per kilogram or in percent, combined with the conversion factor used (i.e. 6,25), or, if the repeatability has been checked, the final quoted result obtained.

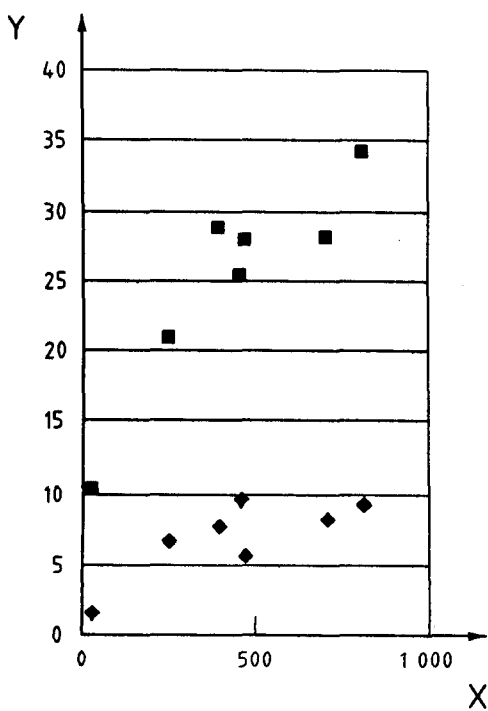
Annex A
(informative)

Results of interlaboratory test

An interlaboratory test was organized by ISO/TC 34/SC 10 in 1987 and carried out in accordance with ISO 5725:1986. The final statistical analysis was carried out in accordance with ISO 5725-2. In this test 25 laboratories participated; samples of corn gluten feed, finished mixed feed stuff, fish meal, mixed feed stuff concentrate (2 types), premixed feedstuff and yeast were investigated.

Table A.1 — Statistical results of interlaboratory test (recalculated 2002)

Parameter	Sample ^a						
	1	2	3	4	5	6	7
Number of laboratories retained after elimination of outliers	23	23	22	17	23	23	23
Mean crude protein content, % (based on dry matter)	70,5	81,1	45,9	3,0	39,6	47,5	25,3
Repeatability standard deviation (s_r), % crude protein	0,29	0,33	0,34	0,06	0,28	0,20	0,24
Repeatability relative standard deviation, %	0,41	0,41	0,75	1,85	0,7	0,42	0,93
Repeatability limit (r) [$r = 2,8 \times s_r$], % crude protein	0,82	0,92	0,96	0,16	0,78	0,56	0,66
Horrat value ^b	0,3	0,3	0,5	0,9	0,5	0,3	0,7
Reproducibility standard deviation (s_R), % crude protein	1,00	1,22	0,90	0,37	1,03	0,97	0,74
Reproducibility relative standard deviation, %	1,42	1,5	1,96	12,1	2,59	2,03	2,94
Reproducibility limit (R) [$R = 2,8 \times s_R$], % crude protein	2,80	3,42	2,53	1,02	2,88	2,78	2,08
Horrat value ^b	0,7	0,7	1,2	3,8	1,2	0,9	1,3
^a Sample 1: fish meal Sample 2: corn gluten feed Sample 3: yeast Sample 4: premixed feed stuff Sample 5: mixed feed stuff concentrate Sample 6: mixed feed stuff concentrate Sample 7: finished mixed feed stuff.							
^b A Horrat value of 1 usually indicates satisfactory precision, while a value > 2 indicates unsatisfactory precision; i.e. a precision that is too variable for most analytical purposes or where the variation obtained is greater than expected for the type of method employed [6], [7].							



Key

X mean, w_m , g/kg

Y precision values, g/kg

- ◆ Repeatability limit r (% crude protein) = $0,008 w_m + 0,3$
- Reproducibility limit R (% crude protein) = $0,027 w_m + 1,3$

Figure A.1 — Relationship between precision values (r , R) and the mean (w_m)

Bibliography

- [1] ISO 1871, *Agricultural food products — General directions for the determination of nitrogen by the Kjeldahl method*
- [2] ISO 5725:1986, *Precision of test methods — Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests* (now withdrawn)
- [3] ISO 5725-1:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*
- [4] ISO 5725-2:1994, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*
- [5] ISO 6497:2002, *Animal feeding stuffs — Sampling*
- [6] HORWITZ W. Evaluation of methods used for regulation of foods and drugs, *Anal. Chem.*, **57**, 1982, pp. 67A-76A
- [7] PEELER J. T., HORWITZ W. and ALBERT R. Precision parameters of standard methods of analysis of dairy products, *J. AOAC*, **72** (5), 1989, pp. 784-806

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